

Figure 1 **A**

2H7scFv-Ig cDNA and predicted amino acid sequence:

HindIII NcoI
~~~~~

2H7 V<sub>L</sub> Leader Peptide→

1    AAGCTTGCCG CC       M D F Q V Q I F S F L L I S A S  
                         ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

2H7 V<sub>L</sub>→

61    V I I A R G Q I V L S Q S P A I L S A S  
      GTCATAATTG CCAGAGGACA AATTGTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

121   P G E K V T M T C R A S S S V S Y M H W  
      CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

BamHI  
~~~~~

181 Y Q Q K P G S S P K P W I Y A P S N L A
 TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATTT ATGCCCATC CAACCTGGCT

241 S G V P A R F S G S G S G T S Y S L T I
 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

301 S R V E A E D A A T Y Y C Q Q W S F N P
 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

(Gly₄Ser)₃ Linker

361 P T F G A G T K L E I K G G G G S G G G
 CCCACGTTG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

2H7 V_H→

421 G S G G G G S S Q A Y L Q Q S G A E L V
 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

481 R P G A S V K M S C K A S G Y T F T S Y
 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

541 N M H W V K Q T P R Q G L E W I G A I Y
 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTAT

601 P G N G D T S Y N Q K F K G K A T L T V
 CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

661 D K S S S T A Y M Q L S S L T S E D S A
 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

721 V Y F C A R V V Y Y S N S Y W Y F D V W
 GTCTATTTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

TITLE: BINDING DOMAIN-IMMUNOGLOBULIN FUSION PROTEINS

Inv: Jeffrey A. Ledbetter et al. Docket No. 3 0.401

EXPRESS MAIL NO. EL 755733415US

Figure 1 B

BclI
~~~~~human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCCAC CGTGCCCGAGC ACCTGAACTC CTGGGGGGAC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCCT CATGATCTCC CGGACCCCTG AGGTCACATG CGTGGTGGTG

961 D V S H E D P E V K F N W Y V D G V E V  
GACGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGGTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCCTCACCG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGTG CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAAGCCC TCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCACAGG TGTACACCCT GCCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAAGG CTTCTATCCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGCAGCCGG AGAACAATA CAAGACCAGC CCTCCCGTGC TGGACTCCGA CGGCTCCTTC

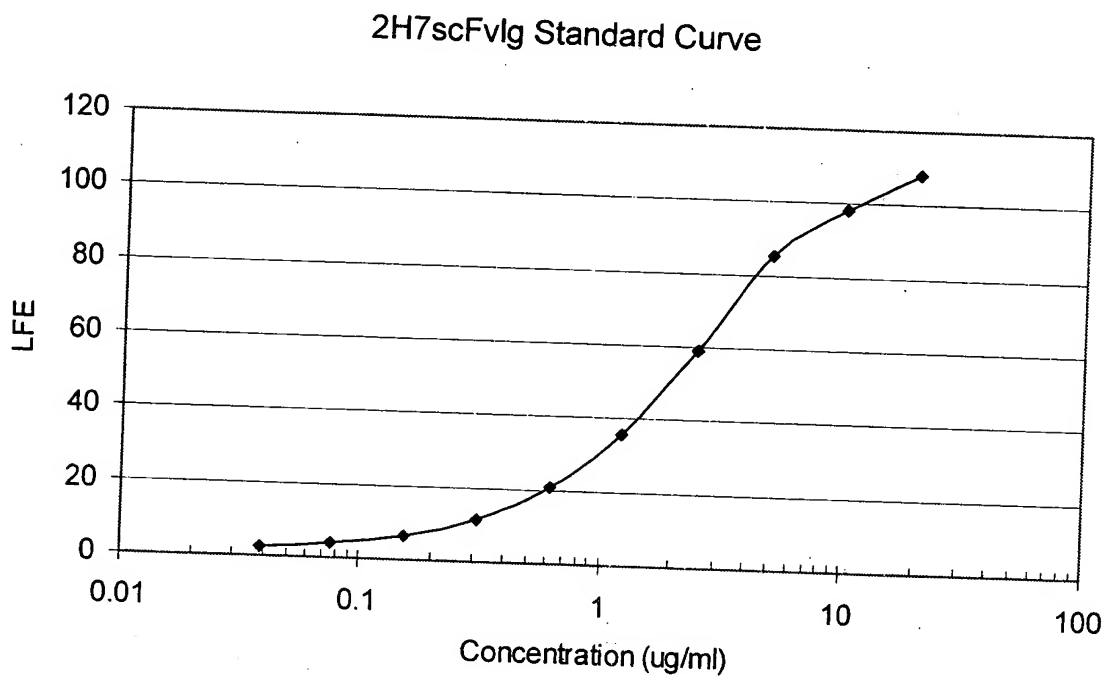
1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCCTCTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441 C S V M H E A L H N H Y T Q K S L S L S  
TGCTCCGTGA TGCATGAGGC TCTGCACAAC CACTACACGC AGAAGAGCCT CTCCCTGTCT

XbaI  
~~~~~

1501 P G K * S R
CCGGGTAAAT GATCTAGA

Figure 2.



Clone	LFE @ 1:50	Estimated Concentration (μ g/ml)
D2	26.1	56
IIIC6	25.7	55
IVA3	28.6	61
Spent bulk	29.6	64

Figure 3.

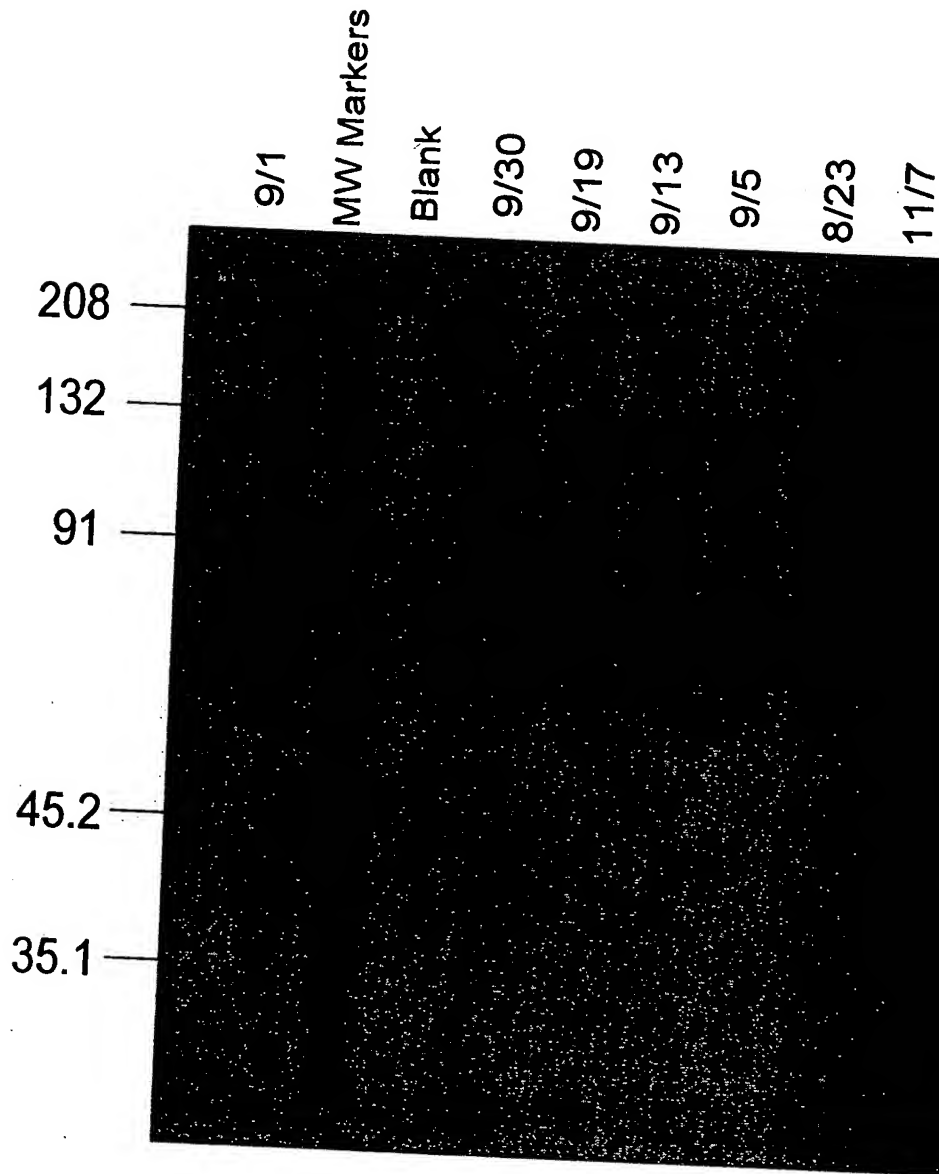


Figure 4A.

Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:

2H7scFv-Ig Concentration	RAMOS	BJAB
20 µg/ml + complement	0.16	0.07
5 µg/ml + complement	0.2	N.D.
1.25 µg/ml + complement	0.32	0.1
Complement alone	0.98	0.94

*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

Figure 4B.

Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:

ADCC Activity of 2H7scFv-Ig on Ramos Cells

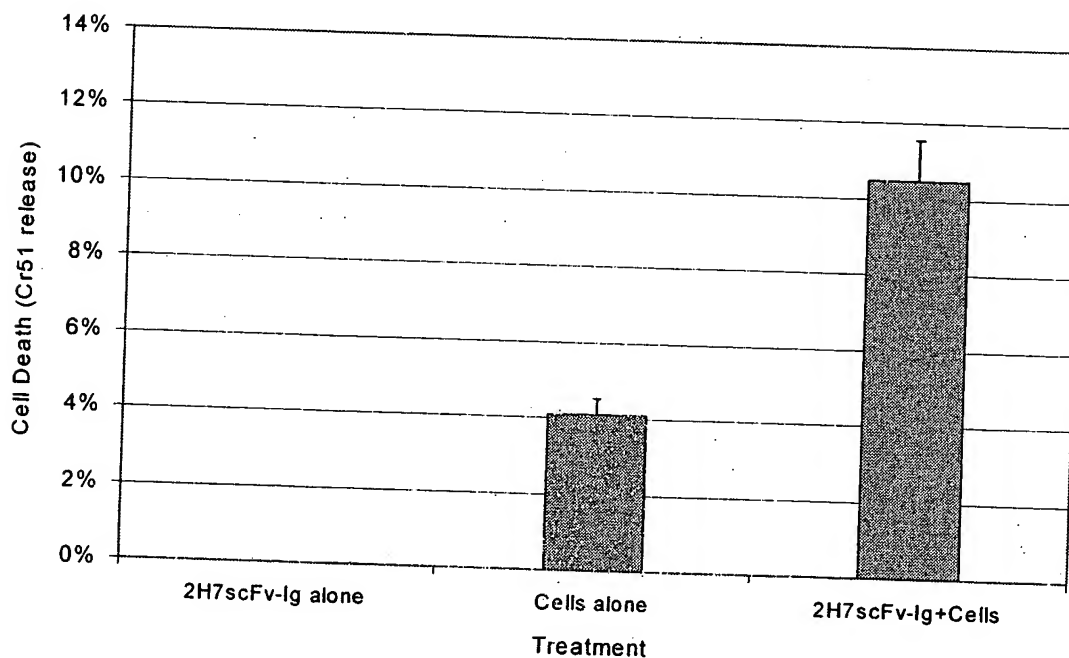


Figure 5.

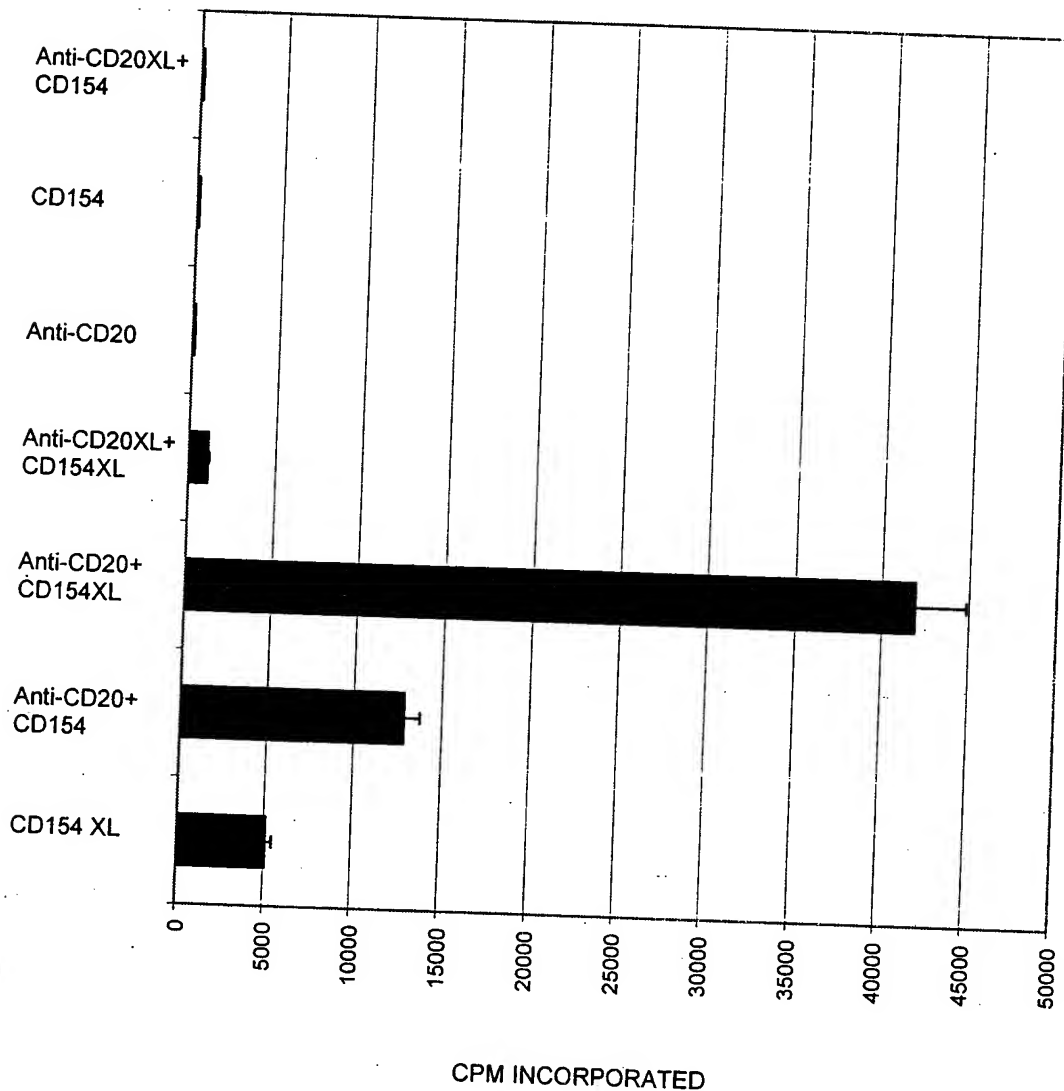


Figure 6A and B.

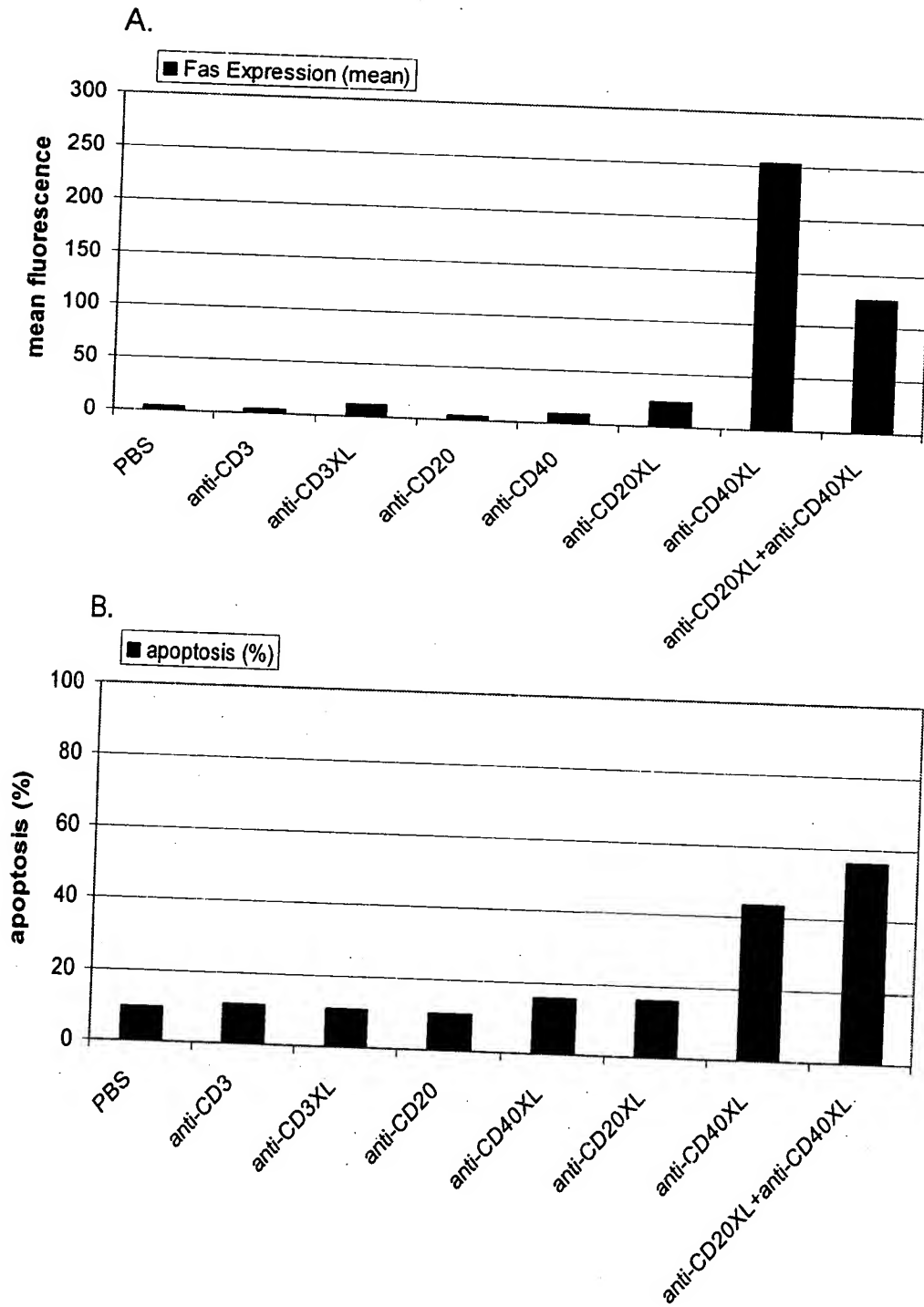


Figure 7A.

2H7-CD154 L2 cDNA and predicted amino acid sequence:

HindIII NcoI 2H7 V_L Leader Peptide →
~~~~~      ~~~~~

1    **AAGCTT**GCCG CC    M D F Q V Q I F S F L L I S A S  
                                 ATGGATTT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                                 2H7 V<sub>L</sub> →

61    V I I A R G Q I V L S Q S P A I L S A S  
GTCATAATTG CCAGAGGACA AATTGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

121    P G E K V T M T C R A S S S V S Y M H W  
CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                 BamHI  
                                 ~~~~~

181 Y Q Q K P G S S P K P W I Y A P S N L A
TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATTT ATGCCCCATC CAACCTGGCT

241 S G V P A R F S G S G S G T S Y S L T I
TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

301 S R V E A E D A A T Y Y C Q Q W S F N P
AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

 (Gly₄Ser)₃ Linker →

361 P T F G A G T K L E L K G G G G S G G G
CCCACGTTG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

 2H7 V_H →

421 G S G G G G S S Q A Y L Q Q S G A E L V
GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

481 R P G A S V K M S C K A S G Y T F T S Y
AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

541 N M H W V K Q T P R Q G L E W I G A I Y
AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT

601 P G N G D T S Y N Q K F K G K A T L T V
CCAGGAAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

661 D K S S S T A Y M Q L S S L T S E D S A
GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG

721 V Y F C A R V V Y Y S N S Y W Y F D V W
GTCTATTCTT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

Figure 7B

human CD154/amino acid 48→

Bcl/Bam hybrid site
781 G T G T T V T V S D P R R L D K I E D E
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAAGGT TGGACAAGAT AGAAGATGAA
841 R N L H E D F V F M K T I Q R C N T G E
AGGAATCTTC ATGAAGATTT TGTATTCATG AAAACGATAC AGAGATGCAA CACAGGAGAA
901 R S L S L L N C E E I K S Q F E G F V K
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG
961 D I M L N K E E T K K E N S F E M Q K G BclI
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT
BclI
~~~~~  
1021 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT  
1081 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTGGT AACCTGGA  
1141 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC  
HindIII  
~~~~~  
1201 F C S N R E A S S Q A P F I A S L C L K
TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG
1261 S P G R F E R I L L R A A N T H S S A K
TCCCCCGGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA
1321 P C G Q Q S I H L G G V F E L Q P G A S
CCTTGCGGGC AACAATCCAT TCACTTGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG
NcoI
~~~~~  
1381 V F V N V T D P S Q V S H G T G F T S F  
GTGTTTGTC AATGTACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT  
XhoI XbaI  
~~~~~  
1441 G L L K L E * * S R
GGCTTACTCA AACTCGAGTG ATAATCTAGA

Figure 7C

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

HindIII NcoI
 ~~~~~  
 ~~~~~2H7 V<sub>L</sub> Leader Peptide→  
 M D F Q V Q I F S F L L I S A S
 1 AAGCTTGCCG CC ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA
 V I I A R G Q I V L S Q S P A I L S A S
 61 GTCATAATTG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT
 P G E K V T M T C R A S S S V S Y M H W
 121 CCAGGGGAGA AGGTCACAAT GACTGTCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG
 BamHI
 ~~~~~  
 Y Q Q K P G S S P K P W I Y A P S N L A  
 181 TACCAGCAGA AGCCAGGATC CTCCCCCAA CCCTGGATT ATGCCCCATC CAACCTGGCT  
 S G V P A R F S G S G S G T S Y S L T I  
 241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTACAATC  
 S R V E A E D A A T Y Y C Q Q W S F N P  
 301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA  
 P T F G A G T K L E L K G G G S G G G  
 361 CCCACGTTG GTGCTGGGAC CAAGCTGGAG CTGAAAGTG GCGGTGGCTC GGGCGGTGGT  
 (Gly<sub>4</sub>Ser)<sub>3</sub> Linker →  
 G S G G G G S S Q A Y L Q Q S G A E L V  
 421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG  
 R P G A S V K M S C K A S G Y T F T S Y  
 481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC  
 N M H W V K Q T P R Q G L E W I G A I Y  
 541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCCTGG AATGGATTGG AGCTATTTAT  
 P G N G D T S Y N Q K F K G K A T L T V  
 601 CCAGGAAATG GTGATACTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA  
 D K S S S T A Y M Q L S S L T S E D S A  
 661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGCG  
 V Y F C A R V V Y Y S N S Y W Y F D V W  
 721 GTCTATTCT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGTCTGG

Figure 7D.

human CD154/amino acid 108 →

781 G T G T T V T V S D P E N S F E M Q K G BclI/Bam hybrid site BclI  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT  
BclI  
~~~~~  
841 D Q N P Q I A A H V I S E A S S K T T S
GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT
901 V L Q W A E K G Y Y T M S N N L V T L E
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT AACCTGGAA
961 N G K Q L T V K R Q G L Y Y I Y A Q V T
AATGGGAAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC
HindIII
~~~~~  
1021 F C S N R E A S S Q A P F I A S L C L K  
TTCTGTTCCA ATCGGGAAGC TTCGAGTCAA GCTCCATTTA TAGCCAGCCT CTGCCTAAAG  
1081 S P G R F E R I L L R A A N T H S S A K  
TCCCCCGGTA GATTGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCCGCCAAA  
1141 P C G Q Q S I H L G G V F E L Q P G A S  
CCTTGCGGGC AACAATCCAT TCACTTGGGA GGAGTATTTG AATTGCAACC AGGTGCTTCG  
NcoI  
~~~~~  
1201 V F V N V T D P S Q V S H G T G F T S F
GTGTTGTCA ATGTGACTGA TCCAAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT
XhoI XbaI
~~~~~  
1261 G L L K L E \* \* S R  
GGCTTACTCA AACTCGAGTG ATAATCTAGA

Figure 8.

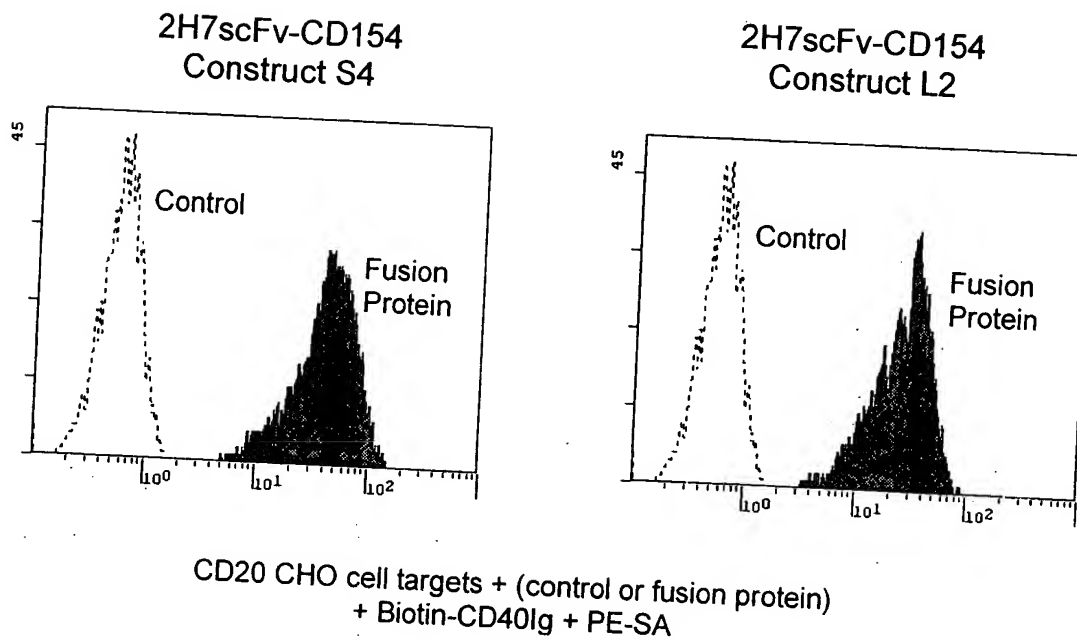
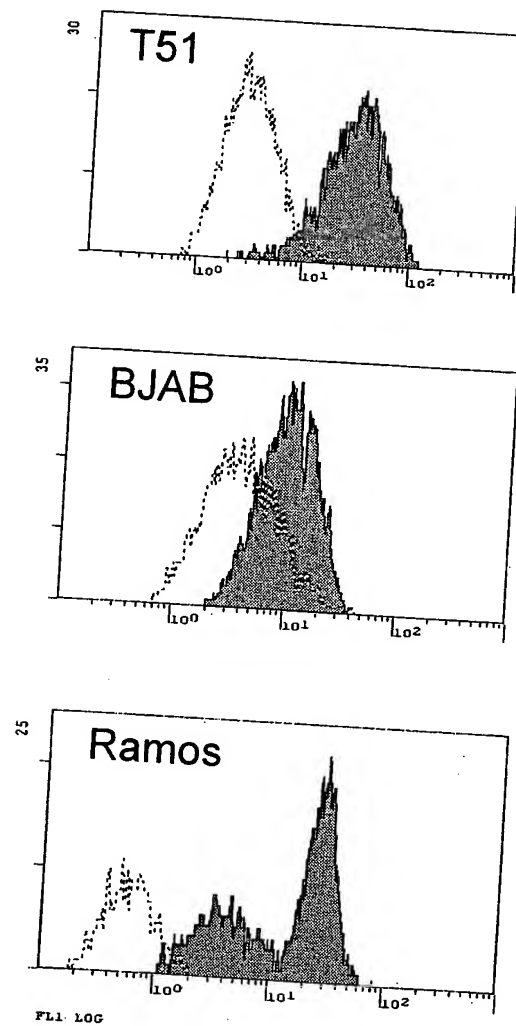


Figure 9.



..... control supernatant    2H7scFv-CD154 supernatant

Figure 10.

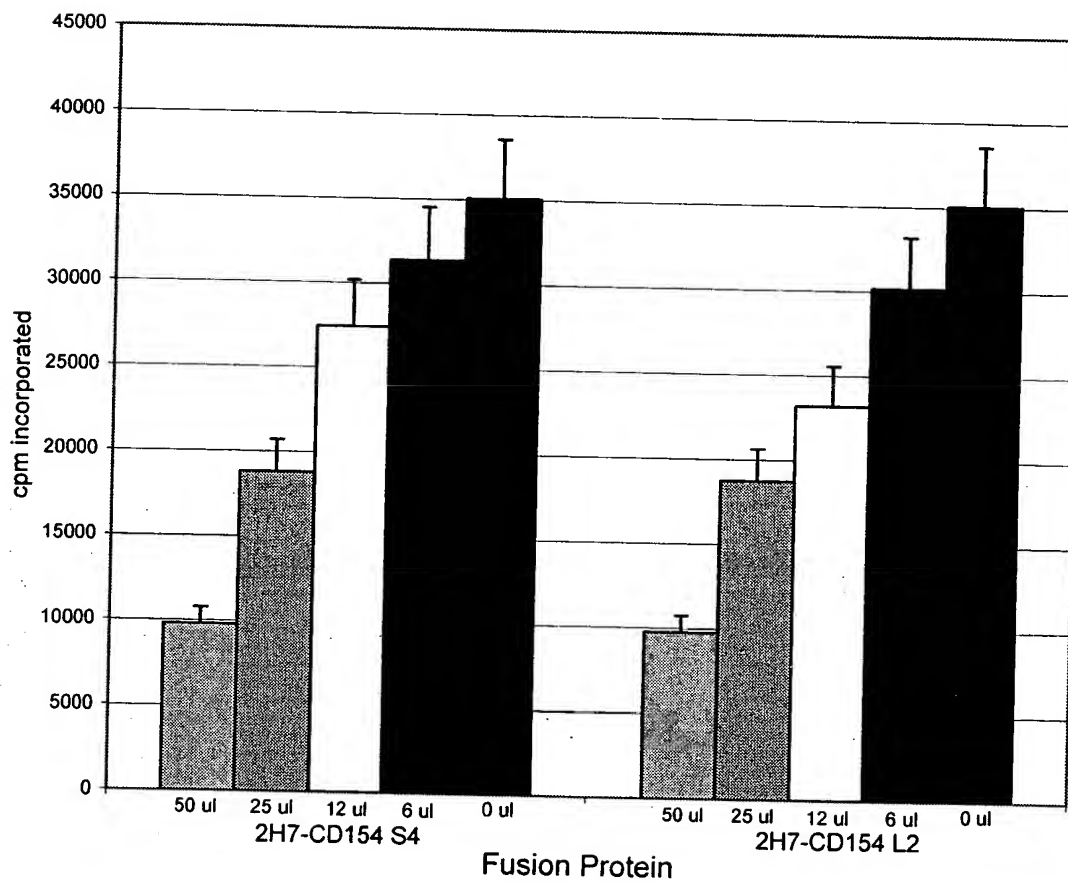
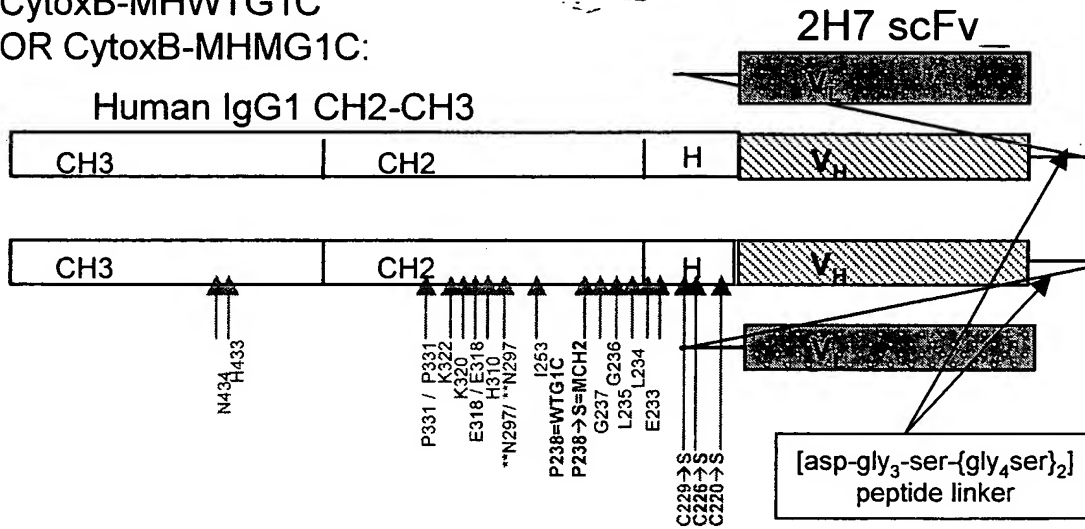
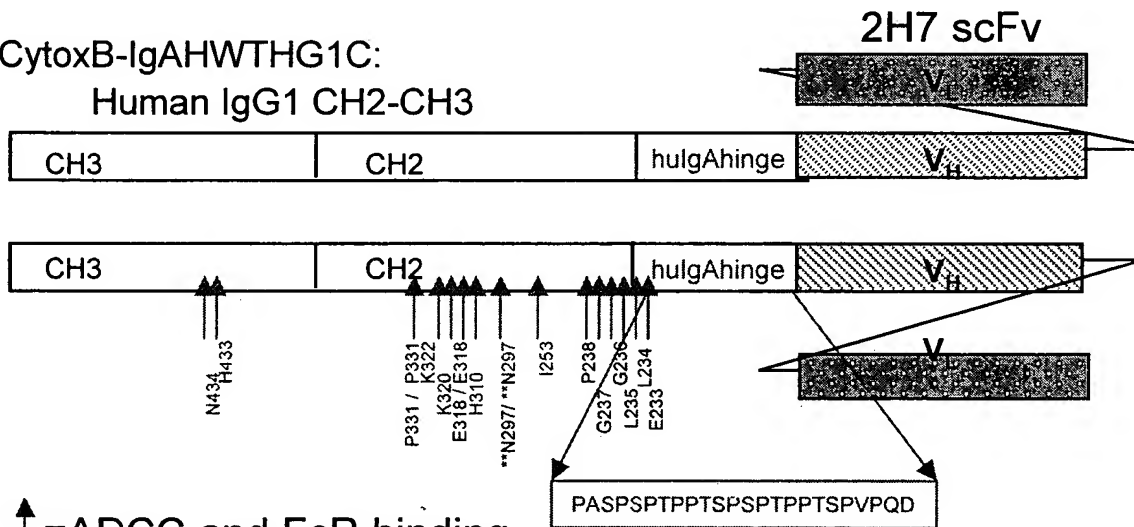


FIGURE 11

CytoxB-MHWTG1C  
OR Cytox-B-MHMG1C:



CytoxB-IgAHWTHG1C:



↑ =ADCC and FcR binding

↑ =Complement Fixation

202700 085900

FIGURE 12

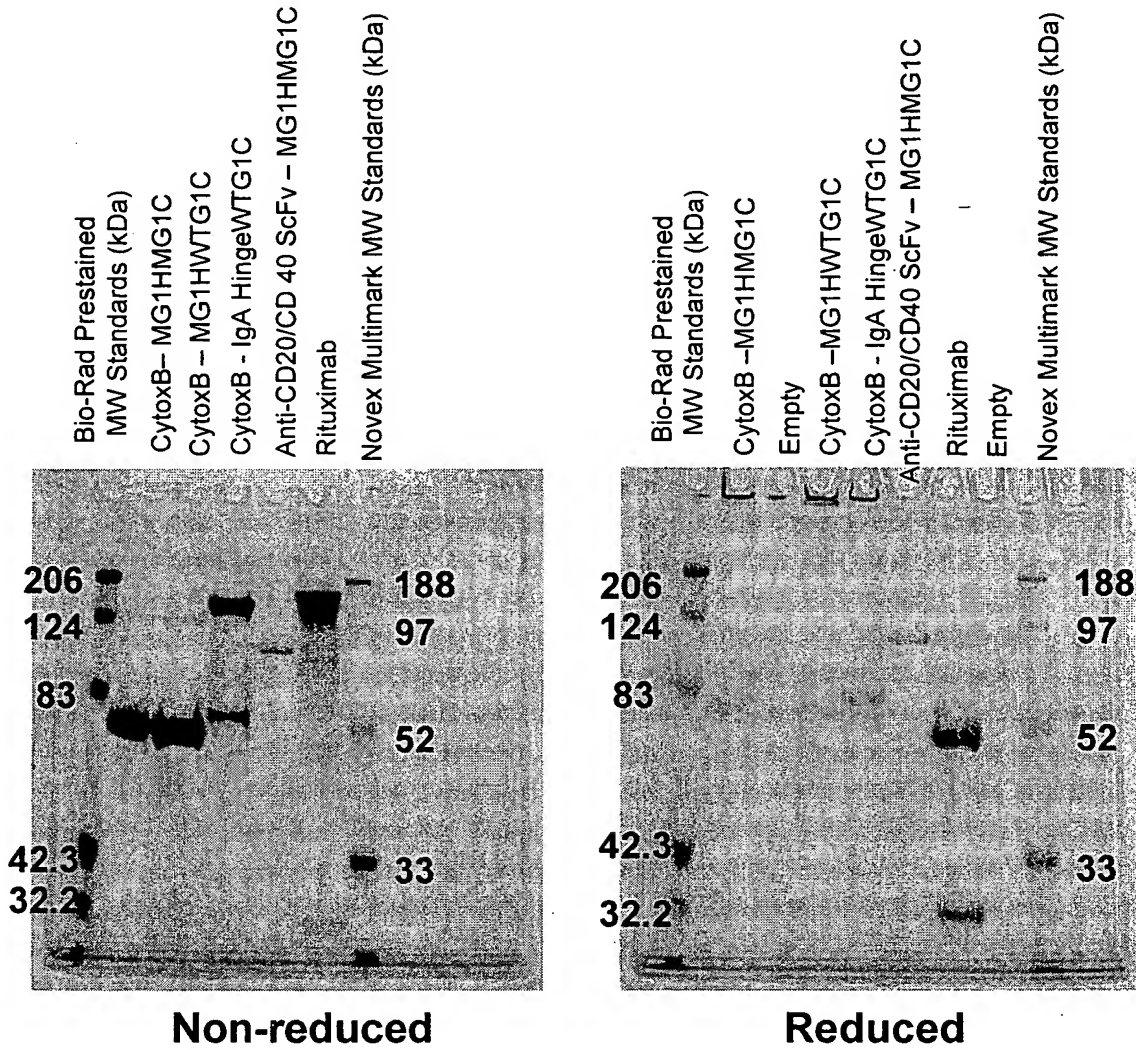




FIGURE 13

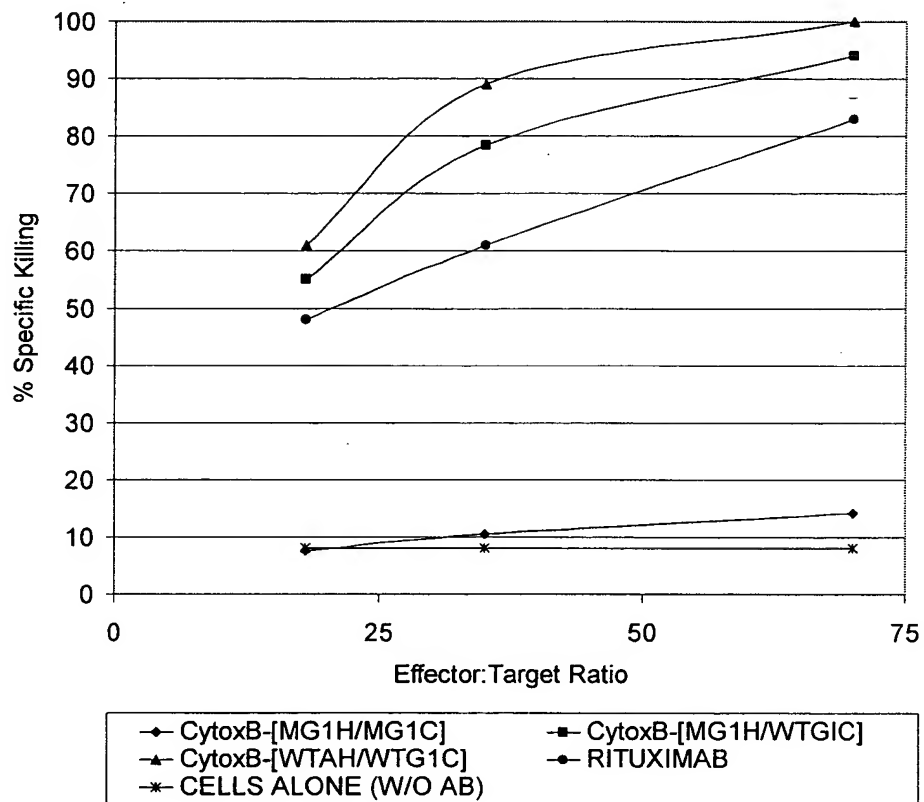
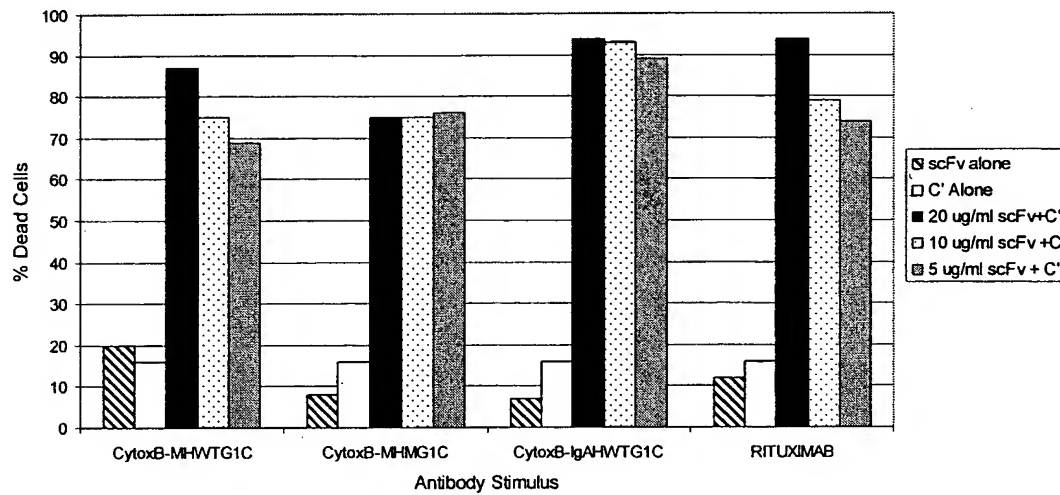


FIGURE 14

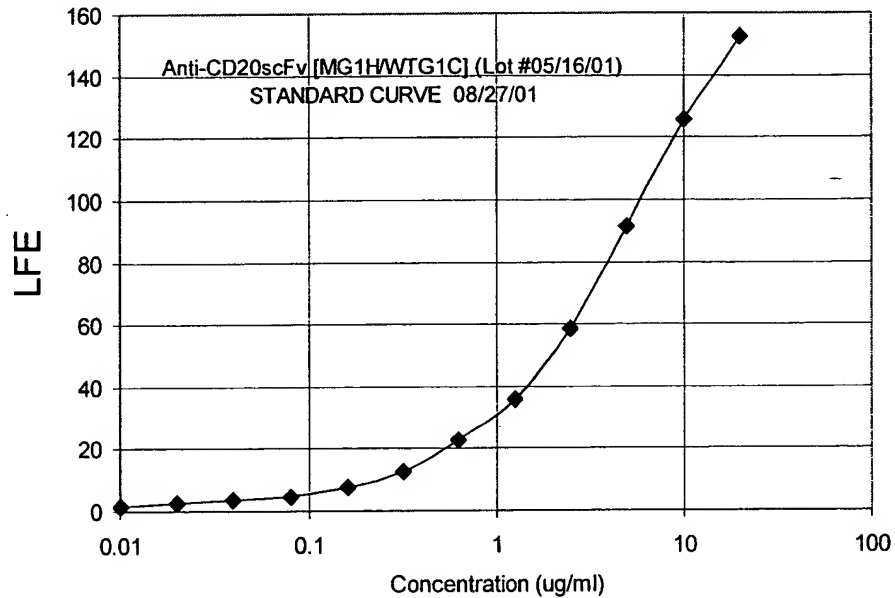


TITLE: BINDING DOMAIN-IMMUNOGLOBULIN FUSION PROTEINS

Investigator: Jeffrey A. Ledbetter et al. Docket No. 300.401

EXPRESS MAIL NO. EL 755733415US

FIGURE 15



		Monkey J99231		Monkey K99334	
Day		LFE(1:40)	Concentration (µg/mL)	LFE(1:40)	Concentration (µg/mL)
Injection →	-7	2.41	<0.6µg/mL	1.51	<0.4µg/mL
	0	2.22	<0.6µg/mL	1.63	<0.4µg/mL
Injection →	1	73.8	220µg/mL	44.4	100µg/mL
	3	20.0	28µg/mL	40.2	80µg/mL
	7	15.6	24µg/mL	15.7	24µg/mL
	8	39.1	80µg/mL	42.6	92µg/mL
	10	11.5	18µg/mL	2.74	1.2µg/mL
	14	2.05	0.6mg/mL	1.96	0.6µg/mL

Figure 16

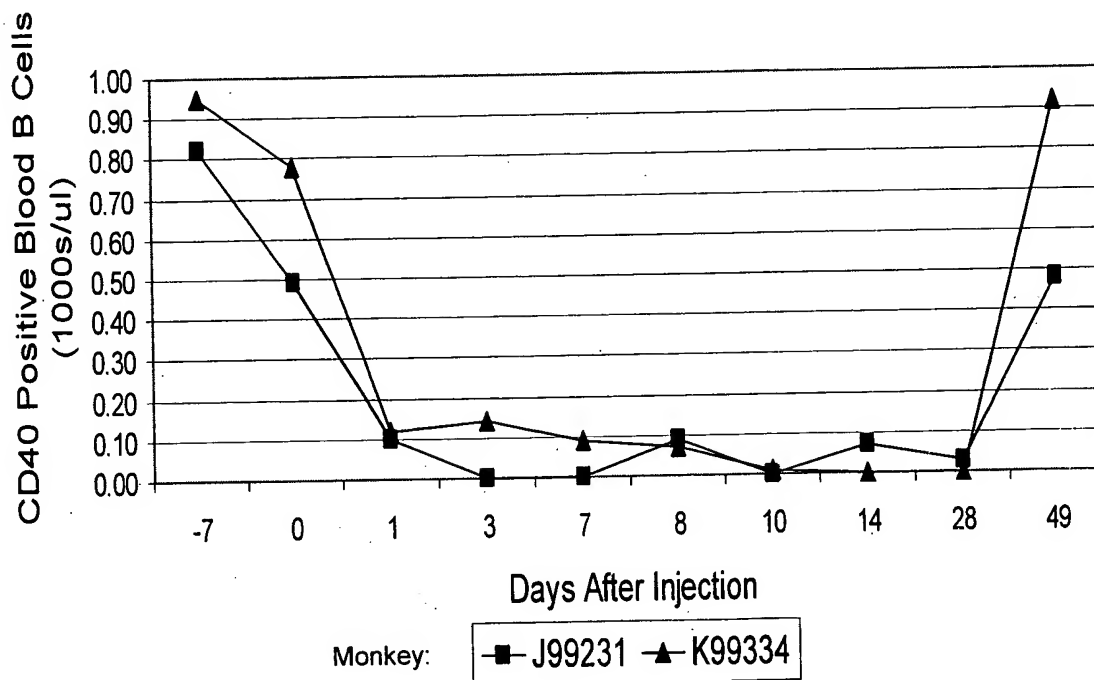
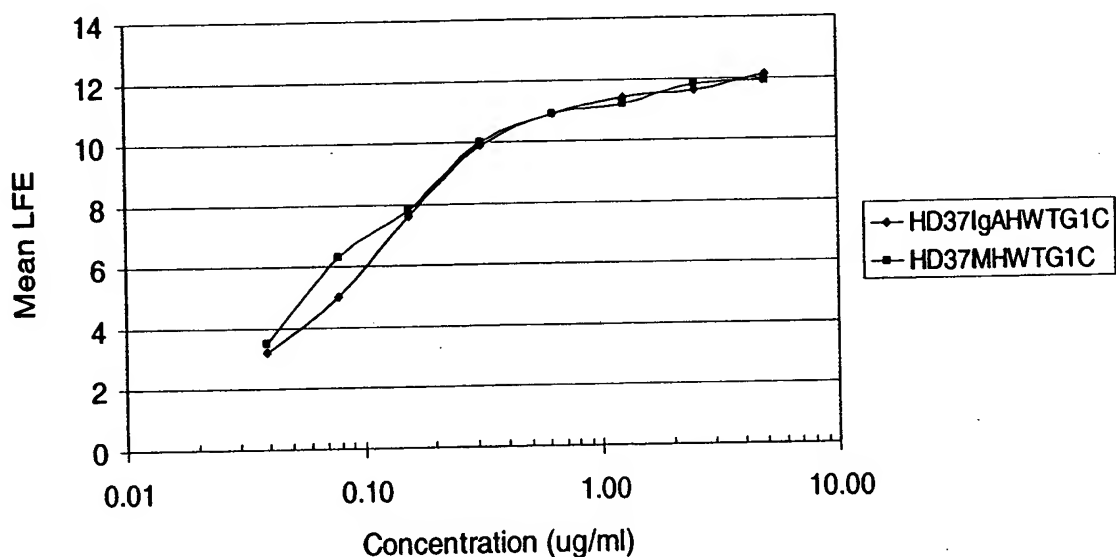


FIGURE 17

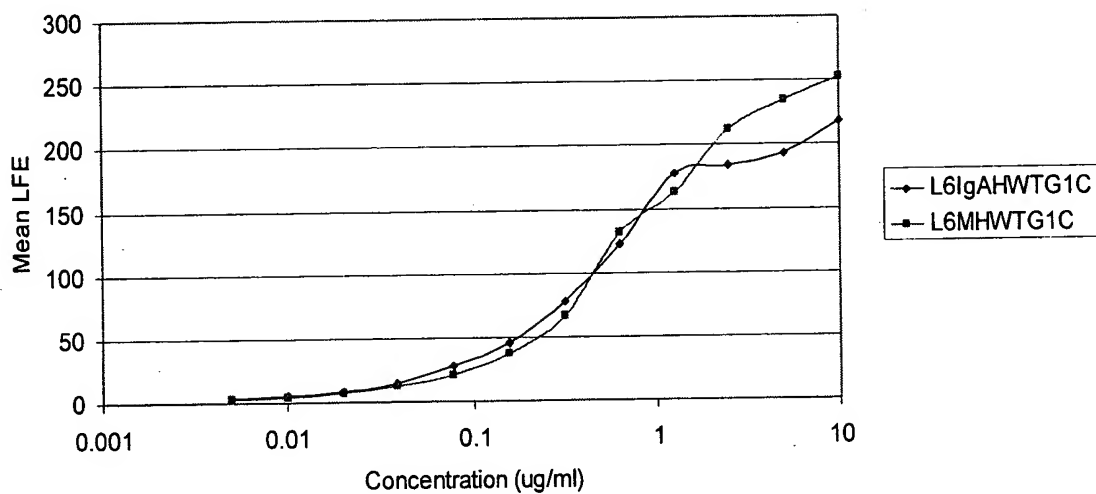
Standard Curve of HD37 scFvlg Derivative  
 Binding to B Cells



Clone/Isolate	Mean LFE at 1:100	Estimated Concentration
Bulk IgAHWTG1C	11.2	> 60 ug/ml
1B2	10.4	>50 ug/ml
6C5	10.5	>50 ug/ml
4B1	8.6	>40 ug/ml
Bulk MHWTG1C	10.9	> 50 ug/ml
2G8	10.6	> 50 ug/ml
3F3	8.3	>40 ug/ml
3D9	11.1	> 60 ug/ml

FIGURE 18

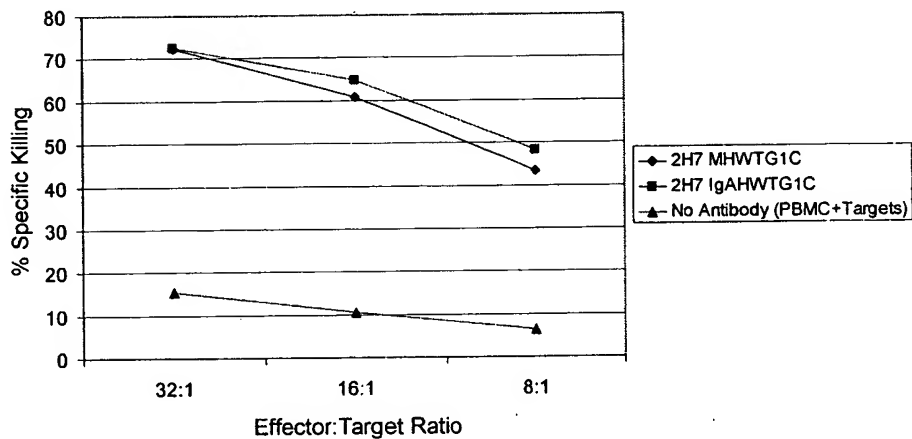
L6 scFvlg Standard Curves



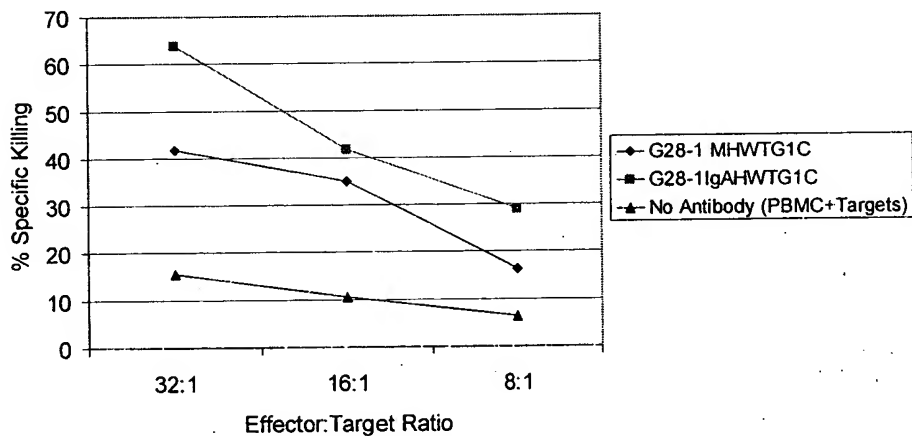
Construct	Mean LFE 1:20	Estimated Concentration
L6IgAHWTG1C unamplified CHO sup	51.1	6.25 ug/ml
L6IgGMHWTG1C unamplified CHO sup	23.0	3.2 ug/ml

FIGURE 19

A. 2H7 (anti-CD20) scFv Derivatives



B. G28-1 (anti-CD37) scFv Derivatives



C. HD37 (anti-CD19) scFv Derivatives

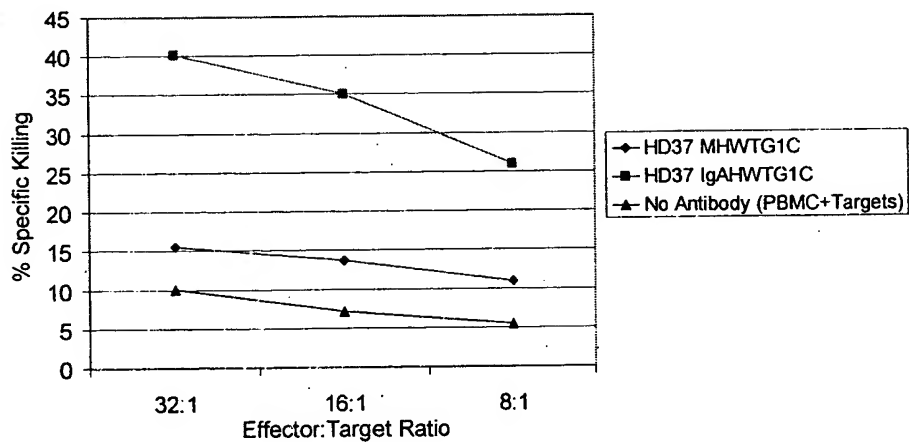


FIGURE 20

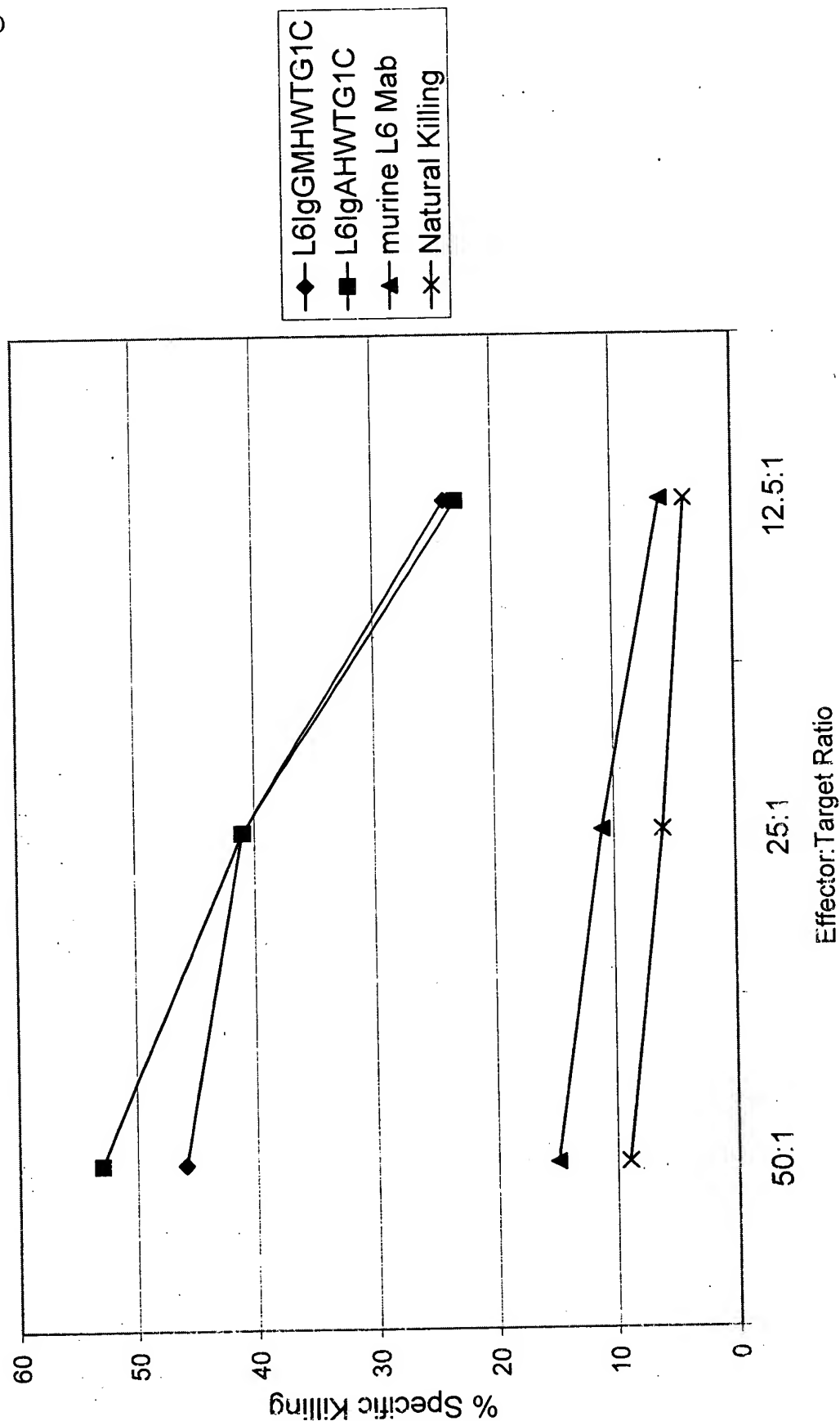




Figure 21

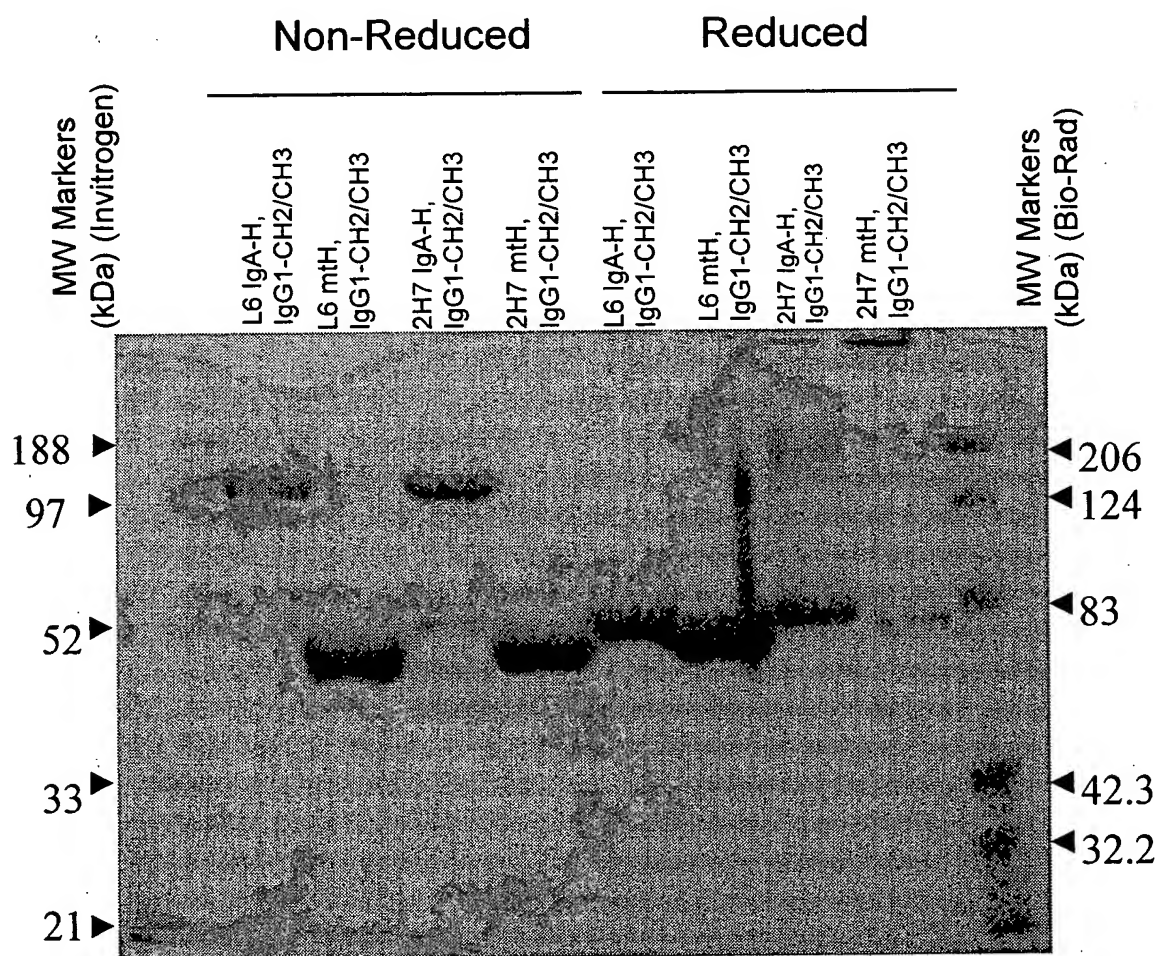


Figure 22

